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A Fragile X Male With a Broad Smear on Southern Blot Analysis Representing 100–500 CGG Repeats and No Methylation at the *EagI* Site of the FMR-1 Gene

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Fragile X DNA studies were carried out on all obligate carriers of a large fragile X family with 10 mentally retarded individuals. One 64-year-old carrier man with an altered FMR-1 allele was not described as being mentally retarded or as having any limitations in function. He was married, raised 8 children, and worked as an auto mechanic. On examination, he had macrocephaly and mild macroorchidism but few of the other typical physical findings of males with fragile X syndrome. His Full Scale IQ is 73, and his Vineland Adaptive Behavior Composite is 73. On the Woodcock–Johnson Psycho-Educational Battery–Revised, he achieved standard scores of 64 in Reading, 55 in Math, and 83 in Knowledge. His DNA findings showed a broad smear on Southern blot analysis of 100–500 CGG repeats and no methylation at the *EagI* site upstream of the FMR-1 protein coding region. His FMR-1 protein production is 12% of normal. His daughters all have large premutations, with somatic instability in the size of the CGG repeat lengths. They all have evidence of academic underachievement and 2 have physical characteristics frequently described in individuals with fragile X.

KEY WORDS: fragile X syndrome, variant mutation pattern, FMR-1 protein, mosaic, high functioning

INTRODUCTION

Individuals with fragile X syndrome are generally described as carrying an altered FMR-1 allele in the full mutation state, but about 15% of males may have variant patterns [de Vries et al., 1993; Hagerman et al., 1994; Rousseau et al., 1994b]. These males are often referred to as mosaics, and they are important to study because they represent the intermediate state between the affected and unaffected male. These males also offer insight into the way this abnormal gene expresses itself clinically. Nolin et al. [1994] reported the largest percentage of mosaics (41%) from their laboratory, but they believed that their techniques led to identification of subtle degrees of mosaicism. They did not find clustering of mosaics in families.

One subgroup of mosaics has large CGG repeat lengths that are within the range of the full mutation, but the mosaics do not have the typical methylation pattern that is associated with the full mutation [Rousseau et al., 1991, 1994b; McConkie-Rosell et al., 1993; Hagerman et al., 1994; Merenstein et al., 1994; Smeets et al., 1995]. An additional mosaic subgroup has CGG repeat lengths that span the premutation and the full mutation ranges, and the DNA will have various amounts of methylation, depending on the lengths of the CGG repeats [Hagerman et al., 1994].

Some initial reports have suggested that males with mosaic CGG repeat length and methylation patterns function at a level similar to males with the full mutation [Rousseau et al., 1991, 1994a; Willems et al., 1992; de Vries et al., 1993], but other reports have suggested that males with mosaic patterns may function at

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higher levels than carriers of the full mutation [Loesch et al., 1993; McConkie-Rosell et al., 1993; Staley et al., 1993; Rousseau et al., 1994b]. Hagerman et al. [1994] reviewed DNA findings on 29 males with fragile X syndrome who had IQs greater than 70. These males represented almost 12% (29/250) of her entire male patient population. Data on CGG repeat length and methylation status were available on 18 patients. Six of those males maintained IQs of more than 70 at age 12. None of these high-functioning males had typical full mutations with more than 200 CGG repeats and fully methylated FMR-1 genes. Three had more than 200 CGG repeats with no methylation of the gene, and their IQs were 100, 94, and 73. They were spared the most serious effect of the condition, which is mental retardation, presumably due to lack of methylation of the gene. The other 3 were mosaics with the premutation portion of their DNA being unmethylated. McConkie-Rosell et al. [1993] described 2 brothers with a broad smear on Southern blot in the range of the full mutation but with only 3% methylation of the *EagII* site of the FMR-1 gene. Both brothers had average to above-average cognitive ability, but their psychological evaluations and physical examinations suggested that they had some manifestations of fragile X syndrome compared with their brother who did not carry the mutation. Smeets et al. [1995] described 2 brothers with large CGG repeat lengths but no methylation of the gene. Physical examinations and detailed cognitive evaluations were not done, but both men were gainfully employed and were believed to function normally. Merenstein et al. [1994] described a high-functioning but emotionally impaired fragile X male with a similar CGG and methylation pattern. Feng et al. [1995b] described a young boy with mild developmental delays and unmethylated CGG repeat lengths that ranged from 100 to 300. In the patient's EBV transformed lymphoblasts, the FMR-1 mRNA levels were normal, but FMR protein (FMRP) production was only 30% of normal. Reduced FMRP was attributed to poor translational initiation efficiency caused by the expanded CGG repeat segment. Individuals described by Smeets et al. [1995], Hagerman et al. [1994], and Merenstein et al. [1994] also had some protein production that may account for their relatively high cognitive functioning. Although information about individuals who display variant DNA patterns is still relatively limited, some males in this intermediate state between a premutation and full mutation function well. Others appear to have substantial manifestations of the disorder.

In addition, males who carry the premutation might not transmit an enlarged and methylated FMR-1 allele to their daughters [Tarleton and Saul, 1993]. Males with the full mutation have sperm with CGG repeat lengths in the range of the premutation [Willems et al., 1992; Reyniers et al., 1993]. One male with CGG repeat lengths in the range of the full mutation and 40% methylation of the gene had a daughter with the premutation [Rousseau et al., 1994b]. Because very few males with the full mutation reproduce, whether their daughters have the premutation is difficult to determine in every case. The man presented in this case study (JT) has 4 daughters, and he transmitted the gene to 3 daughters as very large premutations with somatic instability in the size of the CGG repeat lengths. One daughter's CGG repeat length spanned the premutation/full mutation range (150–250 CGG repeats), but her DNA was entirely unmethylated. Although the mutations in the female offspring were generally smaller than JT's, they overlapped in size, appeared to be unstable, and caused some possible manifestations of the syndrome.

CLINICAL REPORT

JT is a 64-year-old man who was evaluated for fragile X syndrome as part of a family assessment. At the time of the initial home visit, JT did not appear to be cognitively impaired or to have physical characteristics suggestive of fragile X syndrome. However, he later reported that he had quit school during the 4th grade. He was the youngest of 11 children and believes he left school because no one forced him to continue. JT spent much of his adult life working in an automobile shop as a car mechanic. His wife described him as good at his work. He currently drives a tow truck on a part-time basis.

JT married, and he and his wife reared 8 children. His wife described the experience as difficult and stated that, even though her husband always held a job, he often left much of the work around the home to her. His wife also manages the family finances. JT currently lives with his wife, 1 divorced daughter, and her 3 children in a well-kept mobile home. JT is in good health except for cataracts.

Physical Examination

JT (Fig. 1) was 175.5 cm tall (25–50th centile) and weighed 91 kg (90–95th centile); head circumference was 59.5 cm (>98th centile). The head was macro-

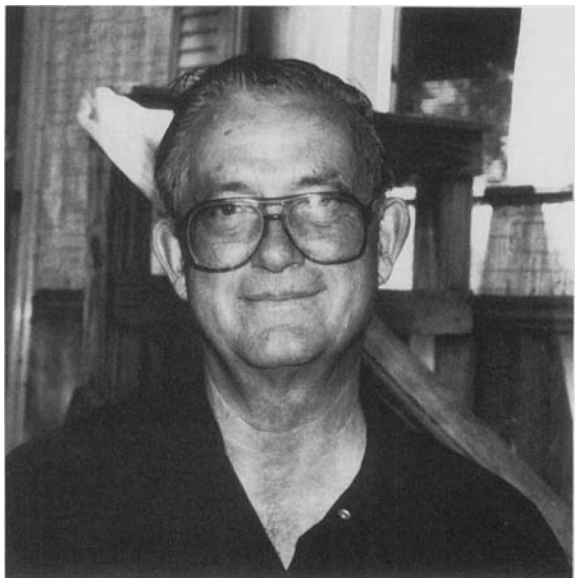

Fig. 1. Index patient.

cephalic. The forehead was not broad, and the face was not elongated. There was no strabismus or nystagmus. A cataract was present on the left eye. Eye contact was appropriate. Both ears measured 7.3 mm (ca. 3 mm larger than the mean). The ears were somewhat simple in configuration and mildly prominent. The palate was highly arched. The chest had a normal configuration, with a mild pectus excavatum. The cardiac examination revealed a grade I–II/VI systolic murmur at the lower left sternal border. The genitalia were normal in appearance, with enlarged testicles measuring 42 cc bilaterally. The limbs were normal without clinodactyly, hand calluses, abnormal horizontal palmar creases, flat feet, hallucal creases, or plantar creases. The neurologic status was grossly normal, with normal strength and normal deep tendon reflexes. On an oral-motor examination, JT demonstrated normal tongue movements, but he had difficulty pronouncing "linoleum."

Laboratory Findings

DNA analysis from a peripheral blood sample indicated an abnormal male pattern on Southern blot analysis (Fig. 2). The pattern observed was a heterogeneous expanded mutation in FMR-1. The heterogeneous mutation pattern ranged from approximately 100 to 500 repeats (as estimated from the Southern blot analysis). There was no methylation at the *EagI* site upstream of the FMR-1 protein coding region. The DNA was digested overnight simultaneously with EcoRI and EagI by using the manufacturer's recommended conditions. Hybridization with DNA probe StB12.3 was performed by using the method described by Rousseau et al. [1991] and Oberlé et al. [1991]. FMRP production was approximately 12% of normal by using EBV transformed lymphoblasts and the method described by Feng et al. [1995a,b] (Fig. 3).

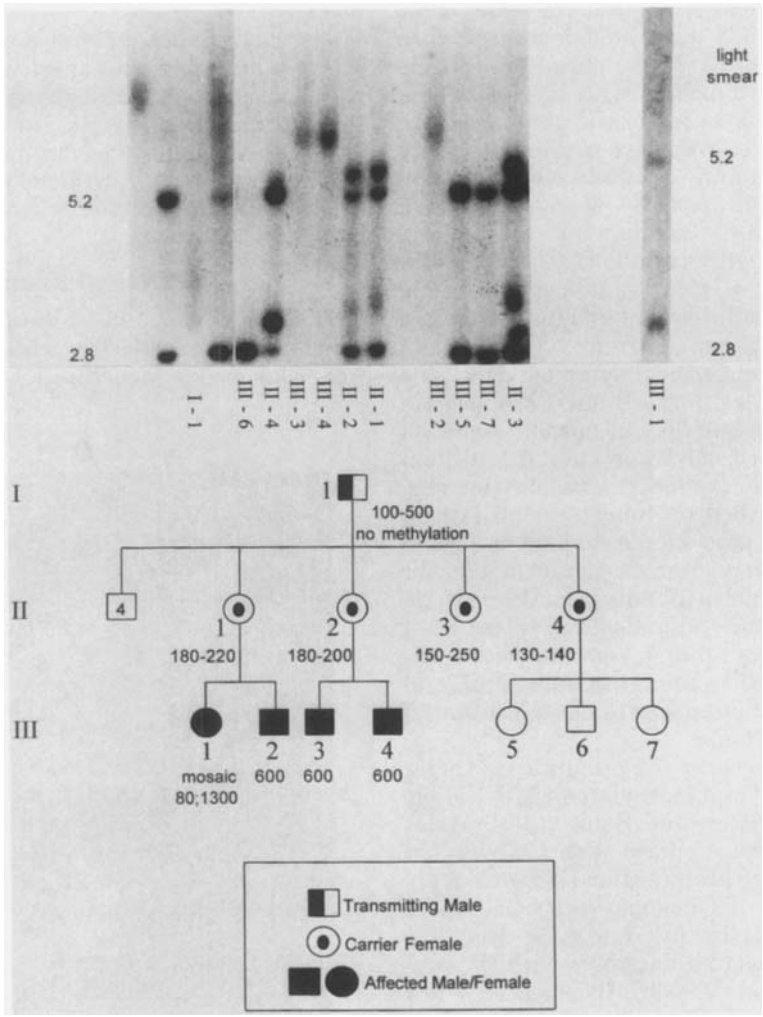

Fig. 2. Southern blot analyses of JT, his children, and grandchildren. The pedigree is included. CGG repeat lengths are included on the pedigree.

Psycho-Educational Findings

Standardized psychological measures were used to assess JT's intelligence quotient and educational skills. JT was cooperative for testing but displayed some anxiety about the testing. On the Wechsler Adult Intelligence Scale–Revised, JT achieved a Verbal IQ of 73, a Performance IQ of 75, and a Full Scale IQ of 73 [Wechsler, 1981]. On the Woodcock–Johnson Psycho-Educational Battery–Revised, standard scores were 64 in Reading, 55 in Mathematics, and 83 in Knowledge [Woodcock and Johnson, 1989]. JT refused the written language evaluation. On the Vineland Adaptive Behavior Scales, standard scores were 34 in Communication, 102 in Daily Living Skills, 100 in Socialization, and 73 on the Adaptive Behavior Composite [Sparrow et al., 1984]. (The mean for all measures is 100 and the standard deviation is 15 points.)

Family History

Three of JT's sibs carried the mutant fragile X allele (Fig. 2). One of JT's sisters has 62 CGG repeats on Southern blot analysis. She does not have obvious manifestations of the fragile X syndrome but does have 1 great-grandson with this disorder. JT's other sibs, who have offspring with fragile X, are deceased.

JT and his wife reared 4 sons and 4 daughters. One son was killed in an automobile accident. One son works in a furniture factory and the other 2 sons work at automobile shops. Two of the 4 sons received high school equivalency certificates (GEDs).

JT's oldest daughter (II-1) completed high school and works in a factory. She lives with her 2 children who have fragile X syndrome. She has a normal appearance except for prominent ears. She describes herself as extremely shy and used to send her sisters into the grocery store for her when she first learned to drive because she was embarrassed when people looked at her. Another daughter (II-4) has strabismus and attended special education classes. She did not finish high school and currently drives a school bus. Another daughter (II-3) was also in a special education program and did not complete high school. She currently works in a cabinet factory. She uses math on her job and reports that her math skills are a relative strength for her. II-2 left school but eventually acquired her high school equivalency certificate. She works in a factory and enjoys bowling in a league. She is married and has 2 children with fragile X syndrome [Spiridigliozzi et al., 1995].

DNA studies on JT's 4 daughters (Fig. 2) revealed typical methylation patterns for premutation females. All had alleles in the upper premutation range, with evidence of somatic instability as demonstrated by smeary autoradiographic signals. Size estimates were 130–140 repeats for II-4, 180–200 repeats for II-2, 180–220 repeats for II-1, and 150–250 repeats for II-3.

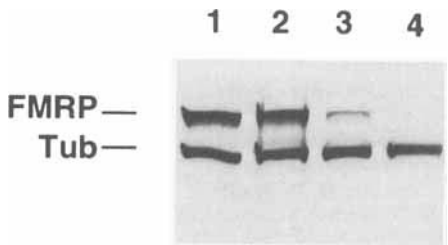

Fig. 3. SDS-PAGE immunoblot analysis of FMRP in EBV transformed lymphoblasts. The immunodetected signals of FMRP and the housekeeping protein beta-tubulin (Tub) are depicted on the left. **Lane 1:** Normal subject with 20 CGG repeats. **Lane 2:** Normal subject with 30 CGG repeats. **Lane 3:** The proband with 100–500 CGG repeats. **Lane 4:** A fragile X subject with approximately 960 CGG repeats. The SDS-PAGE immunoblot analysis was performed as described by Feng et al. [1995a,b]. The FMRP level in the proband is approximately 12% of normal based on the densitometer reading by the same author.

DISCUSSION

Several researchers have reported males with fragile X syndrome who do not carry the typical methylated full mutation. These mosaic males represent about 15% of affected fragile X individuals [de Vries et al., 1993; Hagerman et al., 1994; Nolin et al., 1994]. JT has CGG repeat lengths that span the premutation and full mutation range, but he does not have any detectable methylation. Although JT had borderline scores on IQ testing, he has led a productive life. He is married, has raised a family, works, and reports that he has enjoyed his life. Although he clearly has limitations, he does not consider himself disabled. His wife, who reported some learning difficulties herself, compensated for some of her husband's deficits because she was able to work and manage the family finances. Most or all of JT's deficits are probably caused by the abnormal FMR-1 expression. His physical characteristics, particularly macrocephaly and macroorchidism, are consistent with fragile X syndrome, as is his particular difficulty with mathematics.

JT's daughters have somewhat atypical premutations, with large CGG repeat lengths but no methylation. Based on reports that males who carry the full mutation may have sperm with the premutation [Willems et al., 1992; Reyniers et al., 1993; Rousseau et al., 1994b], we might have expected that JT's daughters would have had smaller, more stable premutations and that that they would have had no manifestations of the disorder. Therefore, their DNA findings and learning problems were somewhat unexpected. CGG repeat lengths in JT's sperm were not determined. However, because he has 1 sister with 62 CGG repeats who has a great-grandchild with fragile X, JT's mother may have had an even smaller premutation and JT's sperm might have a small premutation.

Two of JT's daughters were in special education classes but, to our knowledge, none of his sons required special education. This finding raises strong suspicions that the daughters are either mosaics or that their large premutations limit their ability to function normally. One daughter has strabismus, which is seen relatively often in individuals with fragile X syndrome. Another daughter has prominent ears and reported extreme shyness, characteristics that are often associated with the fragile X phenotype. Unfortunately, JT's wife also reports that she had difficulty in school, and some

of the daughters' difficulties may be related to having a mother with learning problems or to growing up in a family with low socioeconomic status [Spiridigliozzi et al., 1995]. Clearly, more families like this will need to be evaluated to understand the effects of atypical FMR-1 alterations.

CONCLUSIONS

JT had a large smear on Southern blot analysis with 100–500 CGG repeats. The FMR-1 gene is unmethylated. He is not mentally retarded but functions in the borderline range of cognitive ability. He has signs of fragile X syndrome: compromised cognitive functioning, macrocephaly, and macroorchidism. Peripheral blood lymphocytes produced about 12% of the normal amount of FMR-1 protein, which presumably has offered him substantial protection from the full manifestation of the disorder. In spite of significant deficits, JT has led a relatively normal life. JT's 4 daughters carry the FMR-1 gene in the premutation state but have large premutations, with evidence of somatic instability. Two have IQs below 85 [Spiridigliozzi et al. 1995], and all have characteristics that suggest some manifestations consistent with the mildly affected state. Unfortunately, their findings include learning difficulties, shyness, prominent ears, and strabismus, which are frequently seen in the general population, and the findings in these daughters could be misleading.

JT is probably spared the full manifestations of fragile X because some of his DNA is in the premutation range and produces FMRP. The remainder of the DNA is unmethylated, and some of this DNA may also produce FMRP. We hope to obtain skin fibroblast cells to investigate this finding further. In JT's case, impaired functioning of the FMR-1 gene is not caused by methylation of the gene but is most likely related to impaired translation efficiency due to the large number of CGG repeats.

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